Gene flow and vertical stratification of pollination in the bat-pollinated liana species *Marcgravia longifolia*

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Abstract

Pollen dispersal is a key driver of gene flow in plant populations, shaping their spatial genetic structure (SGS). In tropical forests, plant-pollinator interactions vary across vertical strata due to differences in microclimate, resource availability, and foraging behavior. Bats, an important tropical pollinator group, have been observed to exhibit vertical stratification in their foraging activity, with interaction frequencies differing across forest layers. Bats are highly mobile and expected to transport pollen over long distances, but their actual contribution to gene flow has rarely been investigated. *Marcgravia longifolia*, a bat-pollinated Neotropical liana, offers a unique system for studying gene flow across forest strata. Unlike most other plant species, *M. longifolia* produces flowers from the forest floor to the canopy, allowing us to study how bat pollination differs across strata. This study examines pollen dispersal distances, the vertical stratification of gene flow, and SGS in *M. longifolia* at a 100 ha

study site in western Amazonia. Pollen dispersal distances were up to 1350 meters, with longer distances observed in the understory and midstory, where bat foraging activity is more frequent. We detected no SGS, suggesting extensive gene flow facilitated by bat pollination across forest strata. These findings underscore the critical role of bats in shaping plant genetic structure and demonstrate how vertical forest stratification influences gene flow in tropical

Key words: Pollen dispersal, spatial genetic structure, chiropterophily, pollinator-mediated gene flow, forest strata, Amazonian rainforest

1. Introduction

Gene flow, primarily facilitated through pollen and seed dispersal, plays a fundamental role in maintaining genetic diversity within seed plant populations (Browne et al., 2018; Campbell, 1991; Dick et al., 2008). In tropical ecosystems, where over 95% of flowering plant species rely on animal pollinators, biotic vectors such as insects, birds, and mammals are essential to the reproductive success of many plant species (Bawa, 1990; Ollerton et al., 2011). Among these vectors, bats have emerged as particularly effective pollinators in tropical landscapes, offering distinct advantages for plants. Compared to other pollinators, bats transport larger amounts of pollen over longer distances (Fleming et al., 2009; Moreira-Hernández et al., 2021; Muchhala & Thomson, 2010; Wright, 2002). Also, pollen transfer is more targeted, as bats visit a smaller number of species (Diniz et al., 2019; Tschapka & Dressler, 2002). Additionally, plants may reward the small bats with less nectar than non-flying mammals with a similar mobility range (Tschapka & von Helversen, 1999; von Helversen et al., 2003).

Bats occupy specific ecological niches within tropical forests, often exhibiting pronounced vertical stratification across forest strata. While some bat species primarily forage in the understory, others are specialized to forage almost exclusively in the canopy (Bernard, 2001; Gregorin et al., 2017; Kalko & Handley, 2001; Rex et al., 2011; Thiel et al., 2021). This vertical stratification plays a critical role in

structuring biodiversity within tropical forests and significantly influences ecological processes such as pollination (Thiel et al., 2021, 2024).

Marcgravia longifolia J.F.Macbr. (1934) is a woody liana species distributed in western Amazonia (Dressler, 2004; Tropicos, 2024). It offers a unique model for studying gene flow across forest strata due to its flowering and fruiting along the entire vertical gradient from the understory to the canopy. This species produces flagelliflorous inflorescences on the unbranched stem, all the way from ground level up to the canopy. *Marcgravia longifolia* is pollinated by nectarivorous bats (Thiel et al., 2024). The inflorescences show characteristic adaptations to chiropterophily, such as blooming at night, whitish coloration and large nectaries (Fleming et al., 2009; Tschapka & Dressler, 2002; Vogel, 1958). Each inflorescence consists of 20 to 25 fertile flowers arranged in a circle around three to eight extrafloral saccate nectaries (Tirado Herrera et al., 2003). Nectarivorous bats visit *M. longifolia* flowers mostly in the under- and midstory, driven by the higher abundance of inflorescences in these strata (Thiel et al., 2024). The duration of the flowering period in *M. longifolia* is 1.42 months on average, with a peak in August, and individuals flower with an intermediate level of synchrony (Mainardi et al., in prep). Seeds of *M. longifolia* are dispersed by birds and primates (Thiel et al., 2023; Tirado Herrera et al., 2003).

In this study, we investigate gene flow in *M. longifolia* by assessing pollen dispersal distances, vertical stratification of pollen dispersal, and the spatial genetic structure (SGS). We hypothesize that (1) bats move pollen across the entirety of our study area; (2) gene flow is higher in the under- and midstory in *M. longifolia*, where foraging by nectar-feeding bats is more frequent; and (3) SGS is minimal, given the high dispersal distances expected from diverse pollen (and seed) dispersal vectors. Through this research, we aim to deepen our understanding of the ecological processes that drive gene flow in bat-pollinated tropical plants and clarify how forest stratification influences genetic dynamics within species.

2. Material and methods

2.1. Study site

We collected the genetic samples used in this study at the Estación Biológica Quebrada Blanco (EBQB) in Peru (4°21'S 73°09'W). Mean monthly temperatures range from 25 to 27 °C and annual rainfall in the area is ca. 3000 mm with a clear seasonal pattern (Lüffe et al., 2018; Myster, 2015). The EBQB is mostly covered by high ground terra firme rainforest ("bosque de altura", (Encarnación, 1993)), interspersed with swampy areas. A more detailed description of the study site can be found in Heymann et al. (2021) and Heymann & Tirado Herrera (2021).

2.2. Sample collection

Starting in 2015, we systematically conducted extensive searches for *M. longifolia* in the EBQB area, encompassing all habitats suitable for the species. By 2018, we had located 105 adult *M. longifolia* individuals within an area of approximately 100 ha. All individuals were georeferenced with a Garmin GPSMAP 65s. Since the growth habit is unique in the area and can be easily recognized, we are confident that our efforts successfully located all adult *M. longifolia* individuals in the area. We collected cambium samples from adult *M. longifolia*, except for five individuals that died early during fieldwork. Using a sterilized pocketknife, we scraped the outer layers from a 1x1 cm^2 area of the stem and then abraded the cambium layer. The samples were stored in paper bags on silica gel for up to six months before being shipped to the laboratory.

We collected ripe fruits from 13 *M. longifolia* individuals over two periods: October to December 2018 and October to December 2019. In both periods, we sampled ten of the 13 individuals, resulting in data for both years for seven individuals and data for only one year for six individuals. From each infructescence, we collected one to six ripe fruits, recording the height in meters and associated forest stratum. We marked the infructescences for resampling.

We categorized the forest into three strata with clearly distinct vegetation density: understory, midstory, and canopy (Thiel et al., 2024). We chose to classify the forest strata based on vegetation

structure rather than absolute height, as we assume that nectarivores navigate largely according to structural cues instead of specific heights. Thus, the understory, determined by the height of the surrounding shrub and palm layer, varied in height between 3 and 10 m. The canopy was defined as the space between the first major canopy branch (at 9-12 m) of the host tree and its total height (22-32 m). The midstory encompassed the vertical space between the understory and canopy. A figure showing the height distribution in the sampled *M. longifolia* individuals can be found in Thiel et al. (2024, Appendix S3). Using a single-rope climbing technique, we accessed the infructescences and collected the fruits by hand. In total, we collected 459 fruits (246 in 2018 and 213 in 2019).

Since the seeds of *M. longifolia* are tiny, direct DNA extraction from the seeds was not feasible. We therefore germinated the seeds, partially in the field and partially in a greenhouse. After separating the seeds from the fruit pulp, seeds from each fruit were placed in an individually labeled pot. Within 2-3 months, the seeds developed cotyledons, which were then dried and stored on silica gel for up to 12 months.

2.3. DNA extraction

We extracted DNA from the dried cotyledons and cambium using a modified ATMAB protocol as described in Dumolin et al. (1995), based on Hewitt et al. (1991). Samples were placed in 2 ml Eppendorf tubes, frozen in liquid nitrogen, and ground with steel beads using a laboratory mill at 25 Hz. We then added 1 ml of preheated ATMAB extraction buffer and 50 µl of DTT, and incubated the tubes at 55 °C for 1 hour, continuously swaying them at 0.83 Hz and turning them every 15 minutes. After a 10-minute cooling period, 400 µl of dichloromethane was added, and the tubes were centrifuged at 4 °C and 13,000 rpm for 10 minutes. The upper phase (~650 µl) was transferred to new 1.5 ml Eppendorf tubes, to which 400 µl of isopropanol was added. The tubes were gently swayed to detach the pellet and left overnight to ensure complete DNA precipitation. After a second centrifugation at 4 °C and 13,000 rpm for 10 minutes, the supernatant was removed, and the tubes were left to dry for 5 minutes. Then 1 ml of 76% ethanol was added to precipitate the pellet, followed by another centrifugation and removal of the supernatant. The tubes were left to dry completely for

at least 5 hours. Finally, 50 µl of 1x TE + RNase was added for samples from adult plants, and 30 µl for seed samples. After overnight dissolution, the tubes were incubated at 37 °C for 30 minutes. The resulting DNA stock solution was stored at -20 °C and diluted as needed before use.

2.4. Microsatellite development

Microsatellites were developed by ecogenics (2018). First, an Illumina TruSeq Nano library was created from one individual and sequenced using an Illumina MiSeq platform with a Nano v2 500 cycles sequencing chip. Paired-end reads that passed Illumina's chastity filter were de-multiplexed, and Illumina adaptor residuals were trimmed. Quality checks were performed using FastQC v0.117 (Andrews, 2018). Paired-end reads were merged using USEARCH v10.0.240 (Edgar, 2010) to reconstruct the sequence. Merged reads were screened with Tandem Repeats Finder v4.09 (Benson, 1999), identifying 8,215 reads containing a microsatellite insert with a tetra- or trinucleotide of at least six repeat units or a dinucleotide of at least ten repeat units. Primer design was performed with Primer3 (Koressaar & Remm, 2007; Untergasser et al., 2012), yielding 4177 suitable microsatellite candidates. After testing for amplification efficiency and polymorphism, twelve primers were selected that exhibited high reliability of amplification and polymorphism.

We initially analyzed all samples at twelve microsatellite loci, organized into five multiplexes based on annealing temperature. We grouped the markers as follows: ML_373830, ML_657826 and ML_741518 (Multiplex 1); ML_220529 and ML_976893 (Multiplex 2); ML_246705, ML_330921 and ML_1311224 (Multiplex 3); ML_252171 and ML_600839 (Multiplex 4); ML_451323 and ML_1369117 (Multiplex 5). To avoid overlapping in the ABI Analyzer, primers within the same multiplex ware labeled with different dyes. The characteristics of the microsatellite markers are listed in Table A1. The PCR protocols are detailed in Table A2. For each sample, we used 2 µl diluted DNA (10 ng/µl) for PCR. After evaluating marker performance, we retained six loci for further analysis. Microsatellite amplification was performed using Biometra TOne thermocyclers (Analytik Jena AG, Jena, Germany).

2.5. Genotyping

We genotyped the samples using capillary gel electrophoresis on an ABI Genetic Analyzer 3500 (Thermo Fisher Scientific, Waltham, MA, USA). Depending on the marker, we mixed 1.5 µl, 2 µl, or 3 µl of PCR product with 9.8 µl Hi-Di Formamide and 0.2 µl GeneScan 600 LIZ dye Size Standard (Thermo Fisher Scientific). Each multiplex solution was transferred into a 96 microwell plate (Kisker Biotech G060/H/1E-OA). We analyzed the results with Gene Marker 2.7.0 (SoftGenetics, 2016) using predefined panels, which we verified to ensure accurate genotyping. Each sample was then reviewed visually to confirm or adjust the scores. If results were unclear, we repeated the PCR and ABI run. After data review, six markers were eliminated due to errors such as allele inconsistencies between known offspring and mothers or irreproducible results.

We assigned paternity by maximum likelihood using Cervus 3.0.7 (Kalinowski et al., 2007, 2010). Cervus analyses genetic data from co-dominant genetic markers, such as microsatellites, assuming that the species is diploid, that markers are autosomal and inherited independently of each other (Marshall, 2014). We first determined the allele frequencies and summary statistics for adults (Table A3) and seeds (Table A4) for all loci, including expected heterozygosity, polymorphic information content (PIC), exclusion probabilities, Hardy-Weinberg equilibrium, and null allele frequency estimates. Then, we simulated a pair of parents and unrelated candidates, generating LOD scores for correctly and incorrectly assigned parents. Using this distribution, we determined a critical LOD score to differentiate true parents from unrelated candidates with predefined confidence levels.

Paternity assignments were made from the pool of successfully genotyped adults, which represent 80 % of the adult *M. longifolia* individuals in the study area, since some sampled adults could not be genotyped successfully. A minimum of three loci was required for inclusion in the analysis, as the probability of identity dropped below 5% with at least three loci. Paternity was assigned using relaxed confidence intervals (80%), with known mothers pre-assigned based on sampling information. The final

dataset included 105 adult *M. longifolia* individuals and 355 seeds, of which 96 adults and 340 seeds were genotyped at a minimum of three loci and included in the analysis.

2.6. Pollen dispersal

We measured the distance between assigned fathers and known mothers of *M. longifolia* seeds. To examine pollen dispersal distances (response variable) in relation to height and stratum (explanatory variables), we used a linear model for height, as the relationship between height and dispersal distance was linear, and the residuals were both homoscedastic and normally distributed. For stratum, we applied a Kruskal-Wallis test, followed by a post-hoc Dunn's test. The analyses and visualizations were performed using R v4.2.2, using packages geosphere, geodist, spatstat and sp (Baddeley et al., 2002; Hijmans, 2010; Padgham & Sumner, 2018; Pebesma & Bivand, 2005; R Core Team, 2022).

2.7. SGS

We used SPAGeDi 1-5d (Hardy & Vekemans, 2002) to characterize the SGS of adult *M. longifolia* individuals at our study site. We assessed the genetic structure using a spatial auto-correlation analysis of genetic relatedness between the individuals, using the kinship coefficient of Loiselle et al. (1995). Additionally, we computed mean jackknifed estimators and jackknifed standard errors over all loci for kinship coefficients. The number of distance classes used was set to five after assessing the data and with the intent of achieving an even spread of pairwise comparisons between all classes. We computed the Sp value and its p-value in order to assess the intensity of SPS (Vekemans & Hardy 2004). The Sp statistic is defined as $Sp = - b / (F_1 - 1)$, with b as the slope of the regression of F_{ij} on $\ln(d_{ij})$, the natural logarithm of the spatial distance between individuals, and F_1 being the average kinship coefficient between individuals of the first distance class (maximum distance 269 m). We included all adult individuals of *M. longifolia* in the analysis for which the geographic coordinates and the genotype from at least one locus was available (n = 97).

3. Results

3.1. Paternity assignment

We assigned paternity to 72 of the 340 seeds (21 %), coming from all 13 sampled mother plants. The assigned fathers were 48 different individuals. For 13 seeds, the assigned father was also the mother. Most of the seeds with assigned fathers were from fruits collected in the understory (n=32), followed by seeds from fruits collected in the midstory (n=24), and only a minority were seeds collected from fruits in the canopy (n=16).

3.2. Pollen dispersal

Pollen dispersal distances ranged from 0 to 1350 m, with a median of 435 m (Q1 = 126 m, Q3 = 721 m). Pollen were dispersed over larger distances at lower heights (negative linear correlation, multiple R^2 = 0.112, p = 0.004; Fig. 1). Accordingly, pollen dispersal distances were higher in the understory and midstory than in the canopy (Kruskal-Wallis test, χ^2 = 9.178, df = 2, p = 0.01; Fig. 2). The median pollen dispersal distance in the understory was 557 m (Q1 = 295 m, Q3 = 798 m), in the midstory 394 m (Q1 = 203 m, Q3 = 766 m), and in the canopy 152 m (Q1 = 0 m, Q3 = 440 m). Post-hoc Dunn's test showed a significant difference between the understory and canopy (Z = 3.01, adjusted p = 0.01), whereas differences between the understory and midstory (Z = 0.90, adjusted p = 1.00) and between the midstory and canopy (Z = 2.15, adjusted p = 0.09) were not significant.

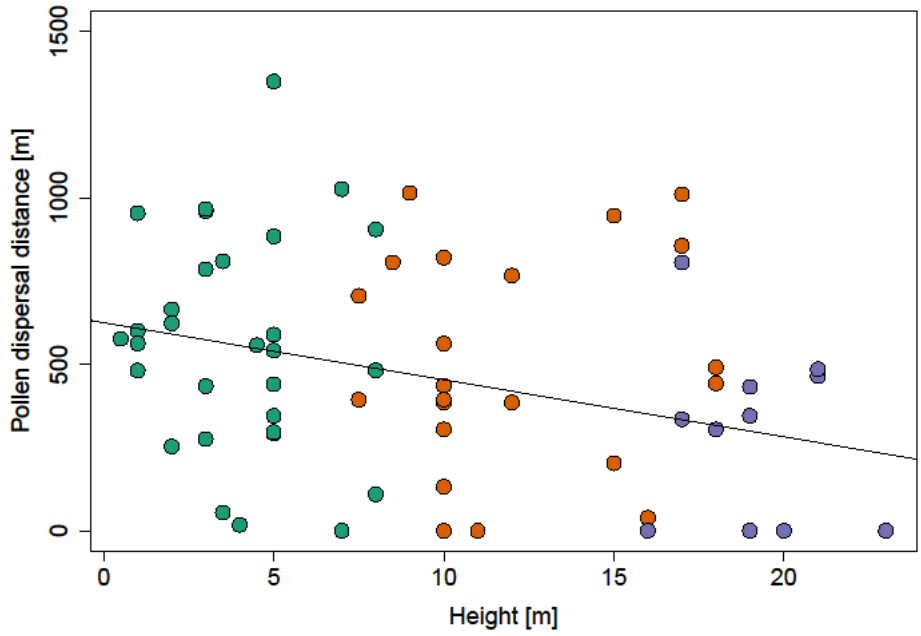

Fig. 1: Relationship between pollen dispersal distance and the height of fruit collection for *M. longifolia* seeds (n = 72) at EBQB. The fitted linear model is: y = 625.188 − 17.139 · x. The model indicates a significant negative relationship (multiple $R^2 = 0.112$, p = 0.004). Green dots represent the understory, orange dots the midstory, and purple dots the canopy.

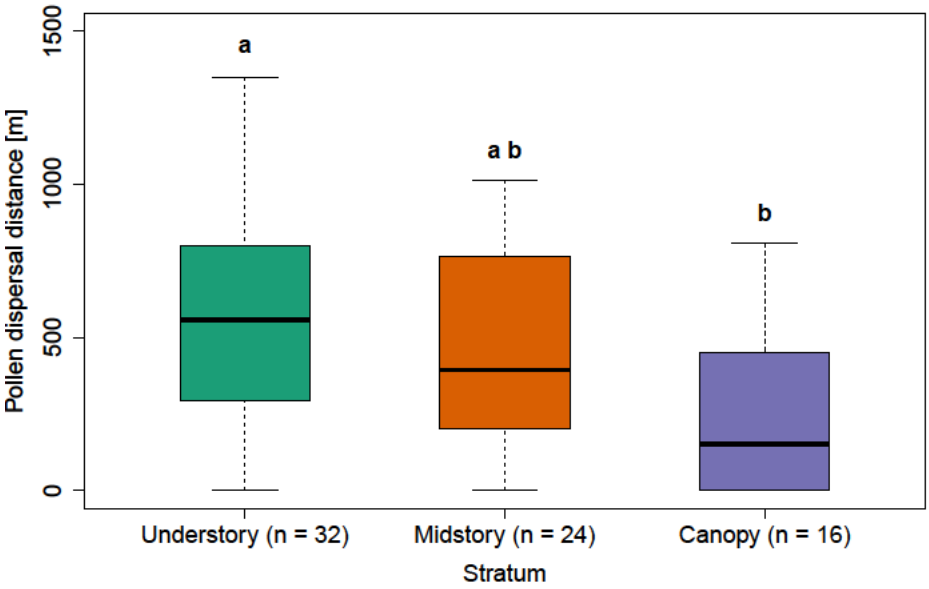

Fig. 2: Pollen dispersal distances between strata for *M. longifolia* seeds (n = 72) at EBQB. Significant differences between strata are indicated by different letters (Kruskal-Wallis test, $\chi^2 = 9.178$, df = 2, p = 0.01). The boxes represent the interquartile range (IQR) for each stratum, with the horizontal line inside the box showing the median value. The whiskers extend from the box to the smallest and largest values within 1.5 times the IQR from Q1 and Q3.

3.3. SGS

We did not detect a SGS for *M. longifolia* (Sp = 0.002 ± 0.004, p = 0.153). Estimates of SGS based on the Sp statistics for the first distance class were $F_{ij(1)}$ = 0.0017, b = -0.002, and r^2 = 0.0002. Autocorrelation showed no signs of a SGS (Fig. 3). Mean distances within distance classes ranged from 170 m for the first class to 988 m for the fifth class.

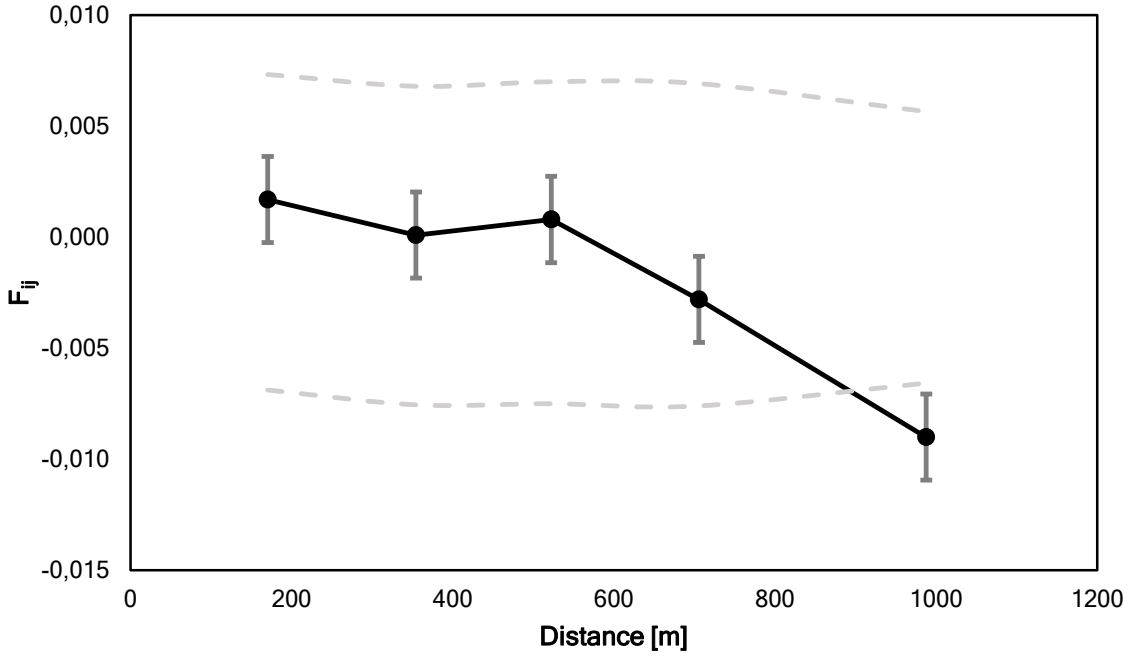

Fig. 3: Average pairwise kinship over pairwise spatial distance [m] for adult *M. longifolia* individuals (n = 97). Pairwise kinship is determined by Loiselle's kinship coefficient (F_{ij}). Dashed lines represent upper and lower 95% confidence intervals.

4. Discussion

We observed that pollen dispersal distances for *M. longifolia* varied widely, with longer distances generally occurring in lower forest strata. We were able to assign paternity to only a small fraction (21%) of the seeds included in our final dataset for paternity analysis. A major limitation was the need to discard half of our 12 initial genetic markers, leaving us with only six loci for genotyping. In many cases, not all six markers were successfully typed, and seeds with fewer than three loci genotyped were excluded, as this was insufficient for reliable paternity assignment. Ideally, we would have used

10 loci to confidently assign paternity to a greater proportion of seeds (Kalinowski et al., 2007), but this was not feasible due to challenges with our study species. Seeds of *M. longifolia* are extremely small and require significant time to germinate and grow, and often left us with very small amounts of leaf material for DNA extraction. This limitation likely affected both the quantity and quality of the extracted DNA. In contrast, DNA extraction from cambium samples of adult plants was much more successful, and we were able to genotype three or more loci for the vast majority of the adult *M. longifolia* individuals.

Although we showed that bats move pollen across the entirety of our study area, the sampling design limited the detection of longer-distance pollen dispersal events, which occurs when bats deliver pollen from donors outside the sampling range. Pollen dispersal distances by bats are highly influenced by environmental factors such as landscape structure and feeding behavior, which affect how far bats transport pollen (e.g. Diniz et al., 2019; Fleming et al., 2009; Horner et al., 1998; Klingbeil & Willig, 2009). Collevatti et al. (2010) reported mean dispersal distances of 132 m for *Caryocar brasiliense*, a bat-pollinated tree in the Brazilian Cerrado, while Biscaia de Lacerda et al. (2008) measured an average pollen flow distance of 827 m for the bat-pollinated tree *Hymenaea courbaril* in the Brazilian Amazon. Maximum pollen dispersal distances depend on the maximum movement distances of the pollen-dispersing bats, which itself is related to the home-range size. Rothenwöhrer et al. (2011) documented home ranges of 12.5 ha for the small nectarivorous bat *Glossophaga commissarisi* in a Costa Rican lowland rainforest. Similarly, Heithaus et al. (1975) found a mean recapture distance of *Glossophaga soricina* of 370 m in a seasonal tropical forest in Costa Rica. These comparisons suggest that the pollen dispersal distances we observed for *M. longifolia* are relatively long, supporting the hypothesis that bats move pollen across the entirety of our study area.

We detected higher pollen dispersal distances in the understory compared to the canopy. This finding aligns with our hypothesis, which was based on previous research investigating foraging visits by nectar-feeding bats at different heights of *M. longifolia* (Thiel et al., 2024). If more bats visit

inflorescences at lower heights, it is likely that pollen dispersal distances in these strata would also be longer. However, the statistical power of our analyses was moderate due to the small sample size.

We found no SGS in *M. longifolia* at our study site. This result supports our hypothesis, as we expected high gene flow due to the overall high mobility and diversity of pollen vectors (at least three bat species, Thiel et al., 2024) and seed dispersers (at least 41 bird species and two primate species, Thiel et al., 2023). Pollen and seed dispersal by these animals occurs over large distances limiting the emergence of a SGS. The continuous habitat at our study site does not present any obvious barriers for bats, further supporting the absence of SGS. Long-range dispersal by both pollen and seed vectors reduces the likelihood of spatial genetic structure (Dick et al., 2008; Gelmi-Candusso et al., 2019).

5. Conclusions

Through this research, we aimed to enhance our understanding of the ecological processes that drive gene flow in bat-pollinated tropical plants, as well as how forest stratification influences genetic dynamics. Our study of the liana *M. longifolia* revealed that bats moved pollen over distances of up to 1,350 m and that pollination distances were vertically stratified within the forest. These findings emphasize the significant role of bats as effective pollen dispersers, contributing to substantial gene flow across the study area. Moreover, given the scarcity of studies on the distance over which bats disperse pollen, our findings offer new insights into the ecological interactions that shape genetic connectivity in tropical rainforest ecosystems.

References

Andrews, S. (2018). *FastQC*. Babraham Bioinformatics.

Baddeley, A., Turner, R., & Rubak, E. (2002). *spatstat: Spatial point pattern analysis, model-fitting, simulation, tests* (p. 3.3-0) [Dataset]. https://doi.org/10.32614/CRAN.package.spatstat

Bawa, K. (1990). Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, *21*, 399–422. https://doi.org/10.1146/annurev.ecolsys.21.1.399

Benson, G. (1999). Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research*, *27*(2), 573–580. https://doi.org/10.1093/nar/27.2.573

Bernard, E. (2001). Vertical stratification of bat communities in primary forests of Central Amazon, Brazil. *Journal of Tropical Ecology*, *17*, 115–126. https://doi.org/10.1017/S0266467401001079

Biscaia de Lacerda, A. E., Kanashiro, M., & Sebbenn, A. M. (2008). Long-pollen movement and deviation of random mating in a low-density continuous population of a tropical tree *Hymenaea courbaril* in the Brazilian Amazon. *Biotropica*, *40*(4), 462–470. https://doi.org/10.1111/j.1744-7429.2008.00402.x

Browne, L., Ottewell, K., Sork, V. L., & Karubian, J. (2018). The relative contributions of seed and pollen dispersal to gene flow and genetic diversity in seedlings of a tropical palm. *Molecular Ecology*, *27*(15), 3159–3173. https://doi.org/10.1111/mec.14768

Campbell, D. R. (1991). Comparing pollen dispersal and gene flow in a natural population. *Evolution*, *45*(8), 1965–1968. https://doi.org/10.1111/j.1558-5646.1991.tb02702.x

Collevatti, R. G., Estolano, R., Garcia, S. F., & Hay, J. D. (2010). Short-distance pollen dispersal and high self-pollination in a bat-pollinated neotropical tree. *Tree Genetics & Genomes*, *6*(4), 555–564. https://doi.org/10.1007/s11295-010-0271-4

Dick, C. W., Hardy, O. J., Jones, F. A., & Petit, R. J. (2008). Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, *1*(1), 20–33. https://doi.org/10.1007/s12042-007-9006-6

Diniz, U. M., Lima, S. A., & Machado, I. C. S. (2019). Short-distance pollen dispersal by bats in an urban setting: Monitoring the movement of a vertebrate pollinator through fluorescent dyes. *Urban Ecosystems*, *22*(2), 281–291. https://doi.org/10.1007/s11252-019-0825-7

Dressler, S. (2004). Marcgraviaceae. In *Flowering Plant – Dicotyledons. The Families and Genera of Vascular Plants* (Vol. 6, pp. 258–265). Springer.

Dumolin, S., Demesure, B., & Petit, R. J. (1995). Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theoretical and Applied Genetics*, *91*, 1253–1256.

ecogenics. (2018). Marcgravia longifolia—*Microsatellite DNA markers*.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Encarnación, F. (1993). El bosque y las formaciones vegetales en la llanura amazónica del Perú. *Alma Mater*, *6*, 95–114.

Fleming, T. H., Geiselman, C., & Kress, W. J. (2009). The evolution of bat pollination: A phylogenetic perspective. *Annals of Botany*, *104*(6), 1017–1043. https://doi.org/10.1093/aob/mcp197

Gelmi-Candusso, T. A., Bialozyt, R., Slana, D., Gómez, R. Z., Heymann, E. W., & Heer, K. (2019). Estimating seed dispersal distance: A comparison of methods using animal movement and plant genetic data on two primate-dispersed neotropical plant species. *Ecology and Evolution*, *9*(16), 8965–8977. https://doi.org/10.1002/ece3.5422

Gregorin, R., Bernard, E., Lobão, K. W., Oliveira, L. F., Machado, F. S., Gil, B. B., & Tavares, V. D. C. (2017). Vertical stratification in bat assemblages of the Atlantic forest of south-eastern Brazil. *Journal of Tropical Ecology*, *33*(5), 299–308. https://doi.org/10.1017/S026646741700027X

Hardy, O. J., & Vekemans, X. (2002). SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, *2*(4), 618–620. https://doi.org/10.1046/j.1471-8286.2002.00305.x

Heithaus, E. R., Fleming, T. H., & Opler, P. A. (1975). Foraging Patterns and Resource Utilization in Seven Species of Bats in a Seasonal Tropical Forest. *Ecology*, *56*(4), 841–854. https://doi.org/10.2307/1936295

Hewitt, G. M., Johnston, A. W. B., & Young, J. P. W. (1991). *Molecular Techniques in Taxonomy*. Springer Science & Business Media.

Heymann, E. W., Dolotovskaya, S., & Tirado Herrera, E. R. (2021). Estación biológica Quebrada Blanco. *Ecotropica*, *23*, 1–4.

Heymann, E. W., & Tirado Herrera, E. R. (2021). Estación Biológica Quebrada Blanco: Un sitio poco conocido para investigación en biodiversidad y ecología en la Amazonía peruana. *Revista Peruana de Biología*, *28*(3). https://doi.org/10.15381/rpb.v28i3.20266

Hijmans, R. J. (2010). *geosphere: Spherical trigonometry* (p. 1.5-20) [Dataset]. https://doi.org/10.32614/CRAN.package.geosphere

Horner, M. A., Fleming, T. H., & Sahey, C. T. (1998). Foraging behaviour and energetics of a nectar-feeding bat, *Leptonycteris curasoae* (Chiroptera: Phyllostomidae). *Journal of Zoology*, *244*(4), 575–586. https://doi.org/10.1111/j.1469-7998.1998.tb00062.x

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2010). Corrigendum. *Molecular Ecology*, *19*(7), 1512–1512. https://doi.org/10.1111/j.1365-294X.2010.04544.x

Kalko, E. K. V., & Handley, C. O. (2001). Neotropical bats in the canopy: Diversity, community structure, and implications for conservation. *Plant Ecology*, *153*, 319–333.

Klingbeil, B. T., & Willig, M. R. (2009). Guild-specific responses of bats to landscape composition and configuration in fragmented Amazonian rainforest. *Journal of Applied Ecology*, *46*(1), 203–213.

Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics*, *23*(10), 1289–1291. https://doi.org/10.1093/bioinformatics/btm091

Loiselle, B. A., Sork, V. L., Nason, J., & Graham, C. (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, *82*(11), 1420–1425.

Lüffe, T. M., Tirado Herrera, E. R., Nadjafzadeh, M., Berles, P., Smith, A. C., Knogge, C., & Heymann, E. W. (2018). Seasonal variation and an “outbreak” of frog predation by tamarins. *Primates*, *59*(6), 549–552. https://doi.org/10.1007/s10329-018-0688-1

Mainardi, A., Kalbitzer, U., Heer, K., Thiel, S., Shahuano Tello, N., & Heymann, E. W. (in prep). Unravelling the phenology of *Marcgravia longifolia* J.F.Macbr. (Marcgraviaceae): Seasonality patterns and climatic cues. *Unpublished Manuscript*.

Marshall, T. C. (2014). *Cervus* (Version 3.0.7) [Computer software]. Field Genetics Ltd.

Moreira-Hernández, J. I., Calderón-Acevedo, C. A., & Muchhala, N. (2021). Fur, wings, and flowers: Development and progress on nectarivorous bat research in the last 50 years. In B. K. Lim, M. B. Fenton, R. M. Brigham, S. Mistry, A. Kurta, E. H. Gillam, A. Russell, & J. Ortega (Eds.), *50 Years of Bat Research: Foundations and New Frontiers* (pp. 135–149). Springer International Publishing. https://doi.org/10.1007/978-3-030-54727-1_9

Muchhala, N., & Thomson, J. D. (2010). Fur versus feathers: Pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist*, *175*(6), 717–726. https://doi.org/10.1086/652473

Myster, R. W. (2015). Flooding × tree fall gap interactive effects on blackwater forest floristics and physical structure in the Peruvian Amazon. *Journal of Plant Interactions*, *10*(1), 126–131. https://doi.org/10.1080/17429145.2015.1029018

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Padgham, M., & Sumner, M. D. (2018). *geodist: Fast, dependency-free geodesic distance calculations* (p. 0.1.0) [Dataset]. https://doi.org/10.32614/CRAN.package.geodist

Pebesma, E., & Bivand, R. (2005). *sp: Classes and methods for spatial data* (p. 2.1-4) [Dataset]. https://doi.org/10.32614/CRAN.package.sp

R Core Team. (2022). *R: the R project for statistical computing*. https://www.r-project.org/

Rex, K., Michener, R., Kunz, T. H., & Voigt, C. C. (2011). Vertical stratification of Neotropical leaf-nosed bats (Chiroptera: Phyllostomidae) revealed by stable carbon isotopes. *Journal of Tropical Ecology*, *27*(3), 211–222.

Rothenwöhrer, C., Becker, N. I., & Tschapka, M. (2011). Resource landscape and spatio-temporal activity patterns of a plant-visiting bat in a Costa Rican lowland rainforest. *Journal of Zoology*, *283*(2), 108–116. https://doi.org/10.1111/j.1469-7998.2010.00748.x

SoftGenetics. (2016). *GeneMarker*. SoftGenetics LLC.

Thiel, S., Gottstein, M., Heymann, E. W., Kroszewski, J., Lieker, N., Tello, N. S., Tschapka, M., Junker, R. R., & Heer, K. (2024). Vertically stratified interactions of nectarivores and nectar-inhabiting bacteria in a liana flowering across forest strata. *American Journal of Botany*, *111*(3), e16303. https://doi.org/10.1002/ajb2.16303

Thiel, S., Tschapka, M., Heymann, E. W., & Heer, K. (2021). Vertical stratification of seed-dispersing vertebrate communities and their interactions with plants in tropical forests. *Biological Reviews*, *96*(2), 454–469. https://doi.org/10.1111/brv.12664

Thiel, S., Willems, F., Farwig, N., Rehling, F., Schabo, D. G., Schleuning, M., Shahuano Tello, N., Töpfer, T., Tschapka, M., Heymann, E. W., & Heer, K. (2023). Vertically stratified frugivore community composition and interaction frequency in a liana fruiting across forest strata. *Biotropica*, *55*(3), 650–664. https://doi.org/10.1111/btp.13216

Tirado Herrera, E. R., Franke, T., Knogge, C., Skrabal, J., & Heymann, E. W. (2003). Flower and fruit visitors of *Marcgravia longifolia* in Amazonian Peru. *Plant Biology*, *5*(2), 210–214. https://doi.org/10.1055/s-2003-40731

Tropicos. (2024). Marcgravia longifolia *J.F. Macbr.* https://www.tropicos.org/name/19800107

Tschapka, M., & Dressler, S. (2002). Chiropterophily: On bat-flowers and flower-bats. *Curtis's Botanical Magazine*, *19*(2), 114–125. https://doi.org/10.1111/1467-8748.00340

Tschapka, M., & von Helversen, O. (1999). Pollinators of syntopic Marcgravia species in Costa Rican lowland rain forest: Bats and opossums. *Plant Biology*, *1*(4), 382–388. https://doi.org/10.1111/j.1438-8677.1999.tb00720.x

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115. https://doi.org/10.1093/nar/gks596

Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*(4), 921–935. https://doi.org/10.1046/j.1365-294X.2004.02076.x

Vogel, S. (1958). Fledermausblumen in Südamerika. *Österreichische Botanische Zeitschrift*, *104*, 491–530.

von Helversen, O., Winter, Y., Kunz, T. H., & Fenton, M. B. (2003). Glossophagine bats and their flowers: Costs and benefits for plants and pollinators. In *Bat ecology* (pp. 346–397). The University of Chicago Press.

Wright, J. S. (2002). Plant diversity in tropical forests: A review of mechanisms of species coexistence. *Oecologia*, *130*, 1–14.

Data Accessibility Statement

The *Marcgravia longifolia* data set, including type (adult or seed), ID of the known mother, seed ID, year of sample collection and genotyping, spatial reference, height class and genotypes for the microsatellites used in the analysis, is deposited on Zenodo (DOI: https://doi.org/10.5281/zenodo.14882215).

Competing Interests Statement

The authors declare no conflict of interests.

Author Contributions

Malika Gottstein: data curation (equal), formal analysis (equal), investigation (equal), resources (equal), writing – original draft preparation (lead), writing – review and editing (equal). Sarina Thiel: data curation (equal), investigation (lead), methodology (equal), resources (lead). Jan Lukas

448 Vornhagen: data curation (equal), formal analysis (equal), investigation (equal), writing – original draft
449 (supporting). Christina Mengel: data curation (lead), investigation (equal), methodology (equal). Marco
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465

466 Appendix

467 Table A1: Characteristics of microsatellite markers for *Marcgravia longifolia*

Primer name	Primer sequences (5'-3')	Repeat motif	T_a [°C]	CoF [µl]	P_{FR} [µl]	Multiplex	Dye
ML252171*	F: AGTCATTTGCATTCTTAGGCG R: AAATCCAAAACCGAGGTGGC	$(TTTG)_{13}$	55.5	1.7	0.95	4	Vic
ML330921*	F: TTAGCCTGTCACTGAGCGTG	$(GGA)_8$	57	2	0.85	3	Pet

	R: TGGTTTCGCCTAAGACTGGG						
ML373830*	F: CAAGCCGATAATGGCACTGG R: CGTCTTTTCCGTAACTGGAGC	$(TCT)_{11}$	58	1.7	0.85	1	Pet
ML657826*	F: TGGATAAGCTTTTGTTGTCGGTC R: ATCACACCGTGCAATTAGGC	$(GA)_{12}$	58	1.7	0.85	1	Ned
ML976893*	F: TGCTCGAAAATGGAAGCCATAC R: CCTCTCTGCAATTATGGACGG	$(TCT)_{8}$	59	1.7	0.95	2	Fam
ML1369117*	F: TCAAGGACGTGATGTTTTGCC R: ACGAGGCATGTTCCCTGTG	$(AAAG)_{7}$	57	1.7	0.75	5	Vic
ML220529	F: GTTCCTTCTTGCTCACTCTAGC R: ACCTTCAACTCCACGCTCTG	$(TC)_{20}$	59	1.7	0.85	2	Vic
ML246705	F: ATATCTGGGGGAGCGCATAG R: GCTACAATTCAGCGTGGGTG	$(AG)_{15}$	57	2	0.85	3	Ned
ML451323	F: ACCGAGTAATTGCGTCTTCTC R: CATTGCGAGGGTCTACGAAC	$(TAT)_{21}$	57	1.7	0.95	5	Fam
ML600839	F: TTGCTAACAAGTCCATGGGG R: TGGGCCGTACATCAATCCTC	$(GA)_{17}$	55.5	1.7	0.95	4	Fam
ML741518	F: AGTAGAGTCCATAAACTAAGGCG R: TGCTATGTTTCACCCAAGCC	$(CTT)_{10}$	58	1.7	0.85	1	Fam
ML1311224	F: CGATGCCCTCACTAGATCCC R: TCTGAGCATCGAAGTGTACG	$(TCT)_{11}$	59	1.7	0.85	3	Fam

468 T_a: annealing temperature, CoF: co-factor $MgCl_2$, P_{FR}: primer (F&R), * used for paternity assignment

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Table A2: PCR protocol used for microsatellite amplification

Component	Volume [µl]		Step	Temperature [°C]	Duration	Repeats
Destilled water	Fill to 14.6	1	Preheat	94		
10x PCR buffer	1.7	2	Initial denaturation	94	5 min	
$MgCl_2$	CoF	3	Denaturation	94	45 sec	35
Primer (F&R)	P_{FR}	4	Primer annealing	T_a	45 sec	35
dNTP mix	1	5	Extension	72	45 sec	35
BSA	0.13	6	Final extension	72	10 min	
Taq	0.13	7	Storage	8		

Table A3: Summary statistics of allele frequency analysis for adult *M. longifolia*

Locus	N	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
ML252171	6	0.761	0.733	0.684	0.681	0.506	0.323	0.118	0.415	NS	-0.0245
ML330921	6	0.593	0.627	0.579	0.780	0.610	0.425	0.187	0.485	NS	0.0308
ML373830	9	0.609	0.672	0.642	0.718	0.530	0.319	0.136	0.450	NS	0.0486
ML657826	11	0.835	0.794	0.760	0.583	0.406	0.221	0.074	0.374	NS	-0.0278
ML976893	5	0.261	0.382	0.361	0.923	0.783	0.633	0.404	0.661	ND	0.1936
ML1369117	7	0.756	0.716	0.674	0.690	0.510	0.318	0.121	0.424	NS	-0.0295
Combined non-exclusion probability					0.142	0.027	0.002	0.00001	0.009		

N: number of alleles, HObs: observed heterozygosity, HExp: expected heterozygosity, PIC: polymorphic information content, NE-1P: non-exclusion probability for first parent, NE-2P: non-exclusion probability for second parent, NE-PP: non-exclusion probability for parent pair, NE-I: non-exclusion probability for identity, NE-SI: non-exclusion probability for sib identity, HWE: deviation from Hardy-Weinberg equilibrium, F(Null): estimated null allele frequency

Table A4: Summary statistics of allele frequency analysis for *M. longifolia* seeds

Locus	N	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
ML252171	8	0.617	0.708	0.656	0.709	0.539	0.357	0.137	0.431	*	0.0701
ML330921	10	0.516	0.596	0.546	0.804	0.643	0.464	0.213	0.506	*	0.0766
ML373830	10	0.438	0.628	0.602	0.758	0.571	0.360	0.164	0.478	***	0.1895
ML657826	14	0.626	0.795	0.769	0.566	0.388	0.199	0.068	0.370	***	0.1113
ML976893	7	0.286	0.386	0.365	0.920	0.780	0.627	0.398	0.657	***	0.1347
ML1369117	5	0.622	0.709	0.665	0.702	0.525	0.338	0.128	0.428	NS	0.0654
Combined non-exclusion probability					0.158	0.031	0.003	0.00002	0.011		

N: number of alleles, HObs: observed heterozygosity, HExp: expected heterozygosity, PIC: polymorphic information content, NE-1P: non-exclusion probability for first parent, NE-2P: non-exclusion probability for second parent, NE-PP: non-exclusion probability for parent pair, NE-I: non-exclusion probability for identity, NE-SI: non-exclusion probability for sib identity, HWE: deviation from Hardy-Weinberg equilibrium, F(Null): estimated null allele frequency